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Deficiency in interferon production by leukocytes from children with recurrent respiratory infections

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Abstract

In vitro interferon production by peripheral blood mononuclear cells from 50 children suffering from recurrent upper respiratory tract infections was examined, and compared with that of 50 healthy children. Five respiratory pathogenic viruses and Mycoplasma pneumoniae were used as inducers. Cells from every child responded to at least three out of the six inducers by interferon production. As a group, cultures prepared from patient cells showed decreased production of IFN when stimulated with adeno, rhino, corona or RS viruses or with the mycoplasma. Similar trend between the two groups of children was seen as regards influenza A virus-induced IFN production in leukocyte cultures. These results corroborate our previous findings that relative deficiency in interferon production appears to be inducer-specific, and suggest that this phenomenon may have a role in the pathogenesis of recurrent respiratory infections.

Key words: Interferon; Child; Respiratory infection

Introduction

Proneness to infection in children is a clinically important problem. Major defects in immune responses, that may bring about a cycle of recurring infections, are rarely demonstrated in these patients. While certain socio-environmental factors are known risk factors for recurring respiratory infections, no unifying biological cause has been discovered for this situation (Lyall et al., 1991). Interferon (IFN) is considered a major factor in host defence against virus infections of humans. Experimental evidence points to the importance of the IFN system among the host resistance factors effective in the early phases of viral infections, and hence, in the limitation of virus spread in tissues. Several studies concerning disturbances of IFN production in various diseases have been reported. Isaacs et al. (1981) were the first to describe some infection-prone children with deficient virus-induced IFN production. The same authors could not reproduce the observation in the same children 2 years later when the children no longer had recurrent infections. This suggested that a genetic

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deficiency was not the reason for the original low IFN yields (Chadda et al., 1984). On the other hand, several other groups have subsequently reported that leukocytes of a proportion of the children suffering from repeated respiratory infections show lowered production of interferon (Bondestam et al., 1984; Pugliese et al., 1985; Vanecek et al., 1985).

All the above studies were carried out using a single "good" inducer virus, either Sendai or Newcastle disease virus (NDV) of the Paramyxoviridae family. We have previously shown that IFN yields from leukocyte cultures stimulated with members of different virus groups vary depending on both the blood donor and the inducer virus (Pitkäranta et al., 1991). Therefore, we considered it worthwhile to reexamine the matter and to use a battery of natural respiratory pathogens as inducers. Interferon production was tested in leukocyte cultures from children with repeated respiratory tract infections and/or with frequent middle ear infections, and from healthy children. The data revealed statistically significant difference in IFN yields when cultures were stimulated with 5 out of the 6 inducers.

Materials and Methods

Patients and controls

The study population comprised fifty consecutive children, aged 8 months to 9 years (median 20 months), admitted during May-August 1992 for scheduled operation to the Department of Otolaryngology, University Hospital of Helsinki, because of undue susceptibility to upper respiratory tract infections or frequent middle ear infections. Inclusion criteria comprised at least 4 acute otitis episodes and/or at least 6 episodes of upper respiratory tract infections during the last 6 months. The patients were enrolled in the study after an informed consent of the parents. History was taken and routine ear, nose and throat examination was performed. As usual, the chain of infections had started at an early age, from 2 months to 5 years (median 6 months). The children had been healthy for at least one week since the last infection, except for 5 patients, who had ongoing mild respiratory tract infections. Four additional patients were under long-term prophylactic sulfisoxazole treatment (from 3 weeks to 3 months) because of recurrent infections. Thirty-nine children had suffered 6 or more episodes of upper respiratory tract infections (rhinitis, pharyngitis or tonsillitis occasionally combined with sinusitis or otitis) within the last 6 months. Eleven children had frequent middle ear infections without associated rhinitis (at least 4 otitis episodes within the last 6 months). During the present visit, adenoidectomy was performed on 48 patients in order to stop the chain of recurrent infections, and ventilation tubes were inserted in 29 patients because of recurrent otitis media. For 10 patients this was a re-operation (re-adenoidectomy and/or re-tympanostomia) because of a continuous chain of upper respiratory tract infections in spite of previous interventions. Blood specimen for leukocyte preparation was drawn immediately before operation using the needle inserted for injecting intravenous anaesthetics.

The control group (50 children, aged 4 months to 2 years, median 12 months) comprised children participating in a trial assessing optimal time schedule for the regular childhood vaccines. They showed no clinical symptoms of disease and had also been previously healthy. Blood for leukocyte preparation was drawn during May–June 1992 when specimens were collected for trial antibody assays (courtesy of J. Eskola and P-R. Rönnberg).

Viruses

Egg-grown Influenza A H3N2 virus (Beijing/353/89) was a gift from R. Pyhälä. Coronavirus 229E was propagated in human embryonic cells, adenovirus 7a in human lung cancer cells (A549), rhinovirus strain 4270 (a local isolate by T. Ziegler) in Hela-Ohio cells, and RSV A3 in Vero cells. Coronavirus and RSV seeds were gifts from T. Ziegler. Aliquots of virus stocks were stored at −70°C. Virus $TCID_{50}$ titres were measured by end-point dilution in tube cultures of the corresponding cells. Mycoplasma pneumoniae (gift from R. Räty) was pelleted from a broth culture, homogenized in a sonicator, suspended in PBS and stored at −70°C as aliquots.

Separation of leukocytes

Leukocytes from heparinized blood were separated over a Ficoll-Isopaque gradient as described earlier (Böyum, 1968). The mononuclear cells at the interphase of the gradient were collected and washed 3 times with phosphate-buffered saline (PBS), adjusted to a concentration of 2×10^6/ml in a tissue culture medium (RPMI-1640 supplemented with 10% fetal calf serum) and distrubuted as 0.1-ml aliquots into 96-well tissue culture plates, 14 wells of each cell batch.

Induction of IFN

Freshly made parallel leukocyte cultures were inoculated, within 6 h of collecting the blood, with samples of 5 crude virus or mycoplasma preparations, 2 wells for each inducer. A single preparation of each inducer was used throughout the study and a fresh aliquot was thawed for each experiment. Influenza-, adeno- and rhinovirus were inoculated at calculated multiplicities of infection (m.o.i.) of 1 was used (Pitkäranta et al., 1988). The titres of the stocks of coronavirus 229E and RSV were too low for this and m.o.i. of 0.01 and 0.1, respectively, were used. Mycoplasma was used at a final concentration of 1 μg/ml (Capobianchi et al., 1987). The plates were sealed with adhesive tape and incubated at 36°C. Culture medium was harvested on day 2 (Pitkäranta et al., 1988), cleared from cells by centrifugation and stored at −20°C until assayed. In pilot experiments all inducers at the selected dosage induced moderate levels of IFN in leukocyte cultures of healthy blood donors.

IFN assay

IFN concentrations were measured by a micromethod based on the reduction of cytopathic effect caused by vesicular stomatis virus (Linnavuori, 1988). A continuous bovine cell line was used in the biological IFN assay. Samples from patient and control cultures were analyzed blindly. A calibrated leukocyte IFN standard (a gift from K. Cantell) was included in all assays so that results could be given in international units (IU). Results given are means of 2 parallel cultures, a maximum of 2-fold difference was seen between the parallel specimens. The subtype of IFN was not specifically assessed in these studies. Virus-induced leukocyte IFN is mostly a mixture of different alpha interferons and bovine cell line used in the assays is not sensitive to human gamma IFN. Therefore, we believe that the IFN yields recorded represent alpha IFN.

Immunoglobulins and complements components

The values were measured by immunoturbidometric method. None of the patients had hypogammaglobulinemia, but one patient had an abnormally high IgG level and a low level of IgA. Three patients had complement C3 values at the lower

borderline of the age-specific range and 2 other patients had similar values for complement C4; otherwise the patients had normal levels of complement components.

Statistical method

Differences between groups were evaluated with the Mann-Whitney U-test.

Results

Leukocytes of 50 children with recurrent respiratory infections and those of 50 controls were examined for interferon production after stimulation with different inducers. Interferon yields from cultures stimulated by adeno-, rhino-, corona-, or RS viruses or mycoplasma were statistically significantly lower for the patient cells than for those of the controls; influenza A virus showed a similar tendency with an almost statistically significant difference (Table I). The uneven distribution of the IFN titres in the two groups of children is shown in Fig. 1. While leukocytes from all children showed interferon production after at least 3 different inducers, 11 of the patients and 2 controls showed no detectable production when stimulated with one or more of the remaining 3 inducers (Table II). Furthermore, leukocytes from as many as 42 of the 50 patients produced an interferon yield below the lower percentile-10 level of the controls when stimulated by at least one of the 6 inducers. Among the 50 controls only 13 children belonged to this low-producer group.

The inducer-specific difference between the patients and controls was supported by a designated mean IFN producing capacity of the children, estimated by calculating a geometric mean value for the yields after the 6 different inducers. Leukocytes from children with recurring respiratory infections showed significantly weaker IFN responses. No difference was noted between the patients with recurrent upper respiratory tract infections and patients with only frequent middle ear infections. The patients with ongoing respiratory tract infection or antibiotic treatment did not differ from the other patients in their in vitro IFN responses to different agents. The production of interferon was influenced by neither the age nor the sex of the children. Spontaneous IFN production was not detected in leukocyte cultures of either patients or controls.

TABLE I

Geometric means of IFN yields produced by leukocyte cultures from children with recurrent infections and control subjects

Inducing agents	IFN IU/ml		*P*
	Patients	Controls	
Influenza A virus	389	622	<0.05
Adenovirus	25	311	<0.001
Rhinovirus	53	215	<0.001
Coronavirus	95	400	<0.001
RS-virus	139	513	<0.001
Mycoplasma	42	196	<0.001

P, statistical significance of the difference assessed by the Mann–Whitney *U*-test.

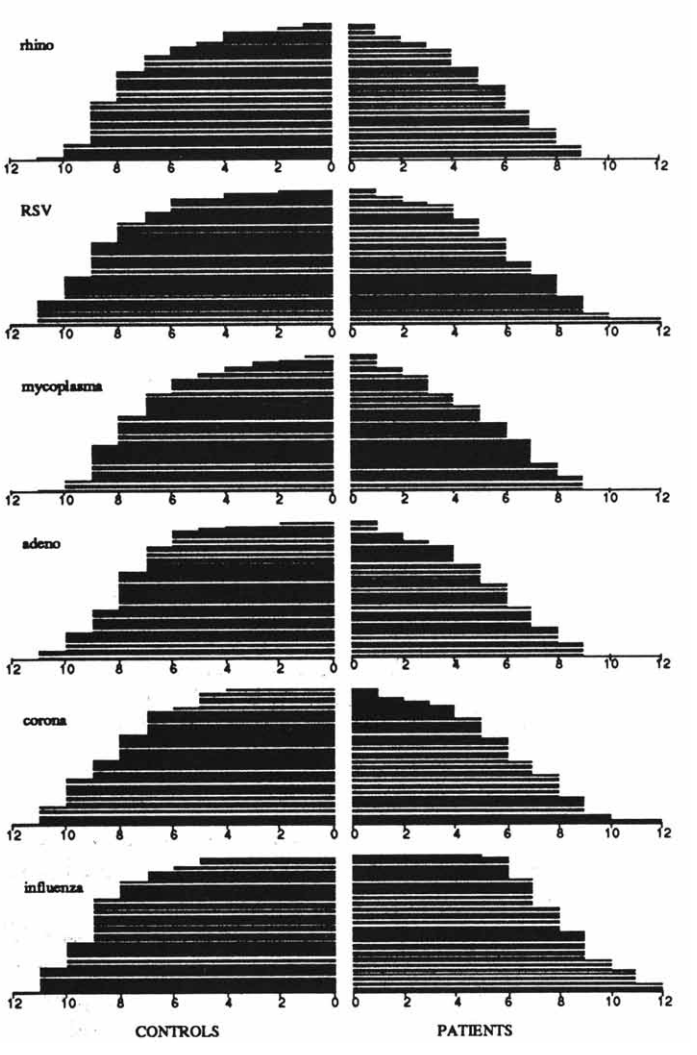

Fig. 1. Distribution of amounts of IFN produced by leukocytes from patients and controls stimulated by different inducers. Culture medium harvested on day 2 was tested. Mean yields of 2 parallel cultures are shown by 50 horizontal bars arranged separately for each inducer according to IFN titre. IFN titres are shown in a logarithmic scale with 1 = <5, 2 = 5, 3 = 10, 4 = 20, 5 = 40, 6 = 80, 7 = 160, 8 = 320, 9 = 640, 10 = 1280, 11 = 2560, 12 = 5120.

Discussion

Reduced virus-induced interferon-α production in leukocyte cultures of some children suffering from recurrent respiratory infections was reported some time ago but evidence accumulated since then has been partly controversial. In this study we have shown that the defect is easily demonstrated by using different natural respiratory pathogens as inducers. Furthermore, the defect is inducer-specific in the sense that a person showing no or a very low response to one or more inducers yet can respond at least moderately to another inducer. Similar variation was also seen in healthy children in accordance with our previous report on adults (Pitkäranta et al., 1991). The defect in interferon production in the patients was demonstrated with 5

TABLE II

Interferon levels (IU/ml) induced by different viruses in leukocyte cultures from selected patients and controls

Children	IFN yields after induction with:					
	Adenovirus	Rhinovirus	Coronavirus	RS-virus	Mycoplasma	Influenza virus
Patients						
1	20	80	320	80	<5	320
2	20	<5	80	160	80	80
3	<5	<5	40	20	<5	80
4	<5	<5	20	80	<5	160
5	10	20	<5	160	20	160
6	20	80	<5	20	<5	160
7	<5	20	160	640	160	640
8	5	<5	5	10	5	80
9	<5	10	20	10	10	160
10	<5	80	160	20	5	320
11	10	40	<5	80	<5	640
Controls						
1	5	<5	20	80	5	80
2	20	<5	40	20	<5	40

All children are listed whose leukocytes failed to produce detectable IFN yields after incubation with one or more of the tested inducers. IFN levels measured in culture supernatants after two days incubation are shown.

out of 6 inducers used. As a result, the calculated mean capacity of IFN production was also lower in the patients than in the controls. The selected 6 inducers represent only a part of common respiratory pathogens.

For the time being it is not possible to judge whether the weak IFN responses represent a primary disorder of interferon production or are due to impaired leukocyte function secondary to the underlying disease. The latter was previously proposed by Pugliese et al. (1985) who found depressed IFN-α production in the winter when children suffered more from recurrent infections. This conclusion was, however, based on observations on 2 children only. Children in our study, both patients and controls, were investigated during summer. Glaser et al. (1986) have shown, by studying leukocytes from medical students during examinations period, that stress can depress concanavalin A-induced (gamma) interferon production. Increased glucocorticoid levels in blood could theoretically affect IFN production (Vanecek et al., 1985). One could speculate that patients had lower interferon production values due to stress preceeding the operation. However, also children in the control group may have been under stress because they were being vaccinated and subjected to venipuncture. Anyhow, stress does not fully explain the inducer-specific difference; thus the putative stress did not significantly affect influenza virus-induced interferon production. Moreover, according to another report, stress can also improve the capacity of leukocytes to produce IFN (Palmblad et al., 1975). Our patients were also slightly older than the controls. However, it is known (Cantell et al.,1968) that age within this range does not affect interferon production, and no tendency towards a correlation between age and IFN yields was seen in this material. From in vitro experiments it is known that cells induced to interferon production show a subsequent refractory

period during which they fail to produce interferon on restimulation. One could propose that our patients were in a refractory period because of a recent infection; a few of them had ongoing infection but they did not differ from the rest of patients in interferon production. Others have also found that acute respiratory infection does not impair IFN production by leukocytes in vitro (Bondestam et al., 1984; Vanecek et al., 1985).

The crucial point in our observations is the inducer-specificity of the lowered IFN response. In principle, such specificity could reflect a genetic property but might also be accounted for by the underlying disease. In the former case, if we assume that the low IFN yields indeed have a role in the decreased resistance to infection, an individual showing poor IFN response to a set of inducers might specifically suffer from infections caused by the respective agents but not from those caused by other agents. In the latter case one assumes that the disease has caused a secondary change in leukocyte subpopulations capable of interferon synthesis. If the former hypothesis is true, one might be able to diagnose the infection-prone children in advance before the chain of infections has started. If the latter alternative is true the responses should return to normal levels in due course after the chain of infections has ceased.

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